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Research Article

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Role of melatonin receptor 1B gene polymorphism and its effect on the regulation of glucose transport in gestational diabetes mellitus

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Abstract: Melatonin receptor 1B (MT2, encoded by the *MTNR1B* gene), a high-affinity receptor for melatonin, is associated with glucose homeostasis including glucose uptake and transport. The rs10830963 variant in the *MTNR1B* gene is linked to glucose metabolism disorders including gestational diabetes mellitus (GDM); however, the relationship between MT2-mediated melatonin signaling and a high birth weight of GDM infants from maternal glucose abnormality remains poorly understood. This article aims to investigate the relationship between rs10830963 variants and GDM development, as well as the effects of MT2 receptor on glucose uptake and transport in trophoblasts. TaqMan-MGB (minor groove binder) probe quantitative real-time polymerase chain reaction (qPCR) assays were used for rs10930963 genotyping. MT2 expression in the placenta of GDM and normal pregnant women was detected by immunofluorescence, western blot, and qPCR. The relationship between MT2 and glucose transporters (GLUTs) or peroxisome proliferator-activated receptor γ (PPARγ) was established by western blot, and glucose consumption of trophoblasts was measured by a glucose assay kit. The results showed that the genotype and allele frequencies of rs10830963 were significantly different between GDM and normal pregnant women (P<0.05). The fasting, 1-h and 2-h plasma glucose levels of G-allele carriers were significantly higher than those of C-allele carriers (P<0.05). Besides, the protein and messenger RNA (mRNA) expression of MT2 in the placenta of GDM was significantly higher than that of normal pregnant women (P<0.05). Melatonin could stimulate glucose uptake and GLUT4 and PPARγ protein expression in trophoblasts, which could be attenuated by MT2 receptor knockdown. In conclusion, the rs10830963 variant was associated with an increased risk of GDM. The MT2 receptor is essential for melatonin to raise glucose uptake and transport, which may be mediated by PPARγ.

Key words: Gestational diabetes mellitus (GDM); Melatonin receptor 1B (*MTNR1B*); Single nucleotide polymorphism (SNP); Glucose uptake; Glucose transporters (GLUTs); Peroxisome proliferator-activated receptor γ (PPARγ)

1 Introduction

Gestational diabetes mellitus (GDM) is a metabolic disorder characterized by abnormal glucose homeostasis, affecting more than one out of seven pregnant women worldwide (Wu et al., 2021; Zhu et al., 2021). GDM is associated with several adverse outcomes for the mother (preeclampsia, cesarean section, metabolic disorders, etc.) and the fetus (macrosomia, neonatal hypoglycemia, metabolic syndrome, etc.) (Pettitt et al., 1985; The HAPO Study Cooperative Research Group, 2008; Bellamy et al., 2009; Fadl et al., 2014; Alharbi et al., 2022). The pathogenesis of GDM is generally thought to be the result of a combination of genetic and environmental factors (Feng et al., 2019). Since GDM and type 2 diabetes mellitus (T2DM) share similar pathological characteristics of β-cell dysfunction, insulin resistance, and abnormal glucose uptake and transport, GDM is also likely to be a polygenic disease (Khan et al., 2014; Powe et al., 2018).

Melatonin receptor 1B (MT2, encoded by the *MTNR1B* gene) is a member of the G protein-coupled

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receptor family. In the past ten years, genome-wide association studies (GWAS) had determined that the variations of *MTNR1B* were closely related to elevated fasting plasma glucose (FPG) and the increased occurrence of T2DM (Karamitri and Jockers, 2019). Moreover, many common single nucleotide polymorphism (SNP) loci of *MTNR1B* gene have been revealed as risk factors of T2DM by several studies (Ling et al., 2011; Patel et al., 2018; Shen and Jin, 2019). Among them, the rs10830963 locus has been widely investigated, and the results indicated that the variation of rs10830963 is a risk factor for T2DM. In addition, rs10830963 variants were also associated with elevated FPG (Karamitri and Jockers, 2019). It is suggested that rs10830963 SNP may be closely associated with glucose metabolism. Considering that abnormal glucose metabolism is one of the pathogenesis factors of GDM, it is necessary to determine its genetic background.

MT2 receptor displays high affinity for melatonin (MLT), a hormone primarily synthesized and released from the pineal gland and other sites of the body, including placental tissue (Soliman et al., 2015). In addition to its important role in regulating circadian rhythms, MLT has also been identified as a key regulator of glucose metabolism. For instance, in a rat model of smoking-induced hyperglycemia, MLT preserves insulin secretion and hepatic glycogen synthesis mediated by the MT2 receptor (Li et al., 2018). The analysis of overexpressed MT2 receptor combined with MLT in skeletal muscle cells indicated the vital role of MT2 receptor for MLT signaling in regulating glucose uptake through activating insulin receptor substrate-1 (IRS-1)/phosphatidylinositol 3-kinase (PI3K) activity (Ha et al., 2006). Thus far, studies on the effect of MLT on the regulation of glucose homeostasis have predominantly focused on pancreatic islets, skeletal muscle, and adipose tissue (Owino et al., 2019; Guan et al., 2021), and research on the effect of MLT on the placenta is scarce. However, the placenta is an important fetal organ during pregnancy, playing a crucial role in regulating maternal and fetal glucose homeostasis. In GDM, maternal hyperglycemia leads to excess blood glucose passing through the placenta, resulting in fetal hyperglycemia. This causes the fetal pancreas to secrete more insulin to eliminate blood glucose, and leads to the storage of excess energy in the form of fat (ACOG, 2020). Therefore, exploring the effect of MLT signaling on glucose transport in the placenta is of particularly importance to understand the impact of maternal GDM on the offspring's glucose metabolism.

Glucose transporters (GLUTs) are facilitative transport proteins with a function to translocate glucose across the plasma membrane, and the expression of GLUT1, GLUT3, and GLUT4 has been previously described in the placenta (Borges et al., 2019; James-Allan et al., 2019; Zhang et al., 2021). GLUTs are located in the syncytiotrophoblast of the placenta, including the maternal-facing microvillous membrane (MVM) and the fetal-facing basal membrane (BM). Net glucose transfer primarily depends on the difference in glucose concentration between the maternal and fetal sides of the placenta, the metabolic level of placental cells, and the expression levels of GLUTs. Notably, placental glucose transport is mainly determined by the intrauterine glucose level and the expression and activity of GLUTs in MVM and BM, which play roles in glucose uptake and glucose transport, respectively (Castillo-Castrejon and Powell, 2017). GLUTs are regulated by multiple signaling pathways. In peroxisome proliferator-activated receptor γ (PPARγ)-deficient 3T3-L1 adipocytes, glucose uptake and the expression levels of GLUT1 and GLUT4 were decreased, suggesting the important role of PPARγ in regulating glucose uptake and transport (Beaumont et al., 2018). However, the effect of MLT signaling on regulating glucose uptake in the placenta remains unclear.

Herein, we set out to assess the relationship between rs10830963 variants in the *MTNR1B* gene and the development of GDM. Then, we further explored the effect of MT2 receptor on the regulation of glucose transport in the presence of MLT in the placenta. Thus, novel targets can be revealed for the prevention of abnormal glucose metabolism in offspring born to mothers with GDM.

2 Results

2.1 Clinical and laboratory parameters of pregnant women

In total, we recruited 856 pregnant women for this study. The mean age of 320 pregnant women in the GDM group was (32.83±4.79) years, while that of 536 pregnant women in the normal pregnancy (NP)

group was (30.32±4.07) years. Compared with the NP group, the GDM group had significantly higher age, pre-pregnancy body mass index (BMI), plasma glucose, and total cholesterol (TC) level (P<0.05). However, pregnant women with GDM had significantly lower plasma triglyceride (TG) level than the NP group (P<0.05; Table 1).

2.2 *MTNR1B* gene polymorphism of pregnant women

The distribution of genotype frequencies in healthy pregnant women was in compliance with the Hardy-Weinberg equilibrium (HWE) (P=0.732). The frequency of CG genotype was the highest compared to CC and GG genotypes (53% for GDM group, 48% for NP group). The frequency of the CC genotype was lower than that of the GG genotype in the GDM group (CC=21%, GG=25%), while that of the CC genotype was higher than that of the GG genotype in the NP group (CC=33%, GG=19%). The frequency of the G allele was significantly higher than others in the NP group (52% for GDM group, 43% for NP group; P<0.05). Moreover, using the three genotype models (dominant model, recessive model, and additive model) for pregnant women, we could find a significant relationship between rs10830963 and the risk of GDM after adjusting for age and BMI (P<0.05). The odd ratios for the three genotype models were as follows: 1.818 (confidence interval (CI): 1.290–2.562) for the dominant model, 1.459 (CI: 1.024–1.648) for the recessive model, and 2.085 (CI: 1.355–3.209) for the addictive model (Table 2).

2.3 Relationships between the genotype frequencies of rs10830963 and the metabolic parameters of pregnant women

We explored the relationship between the variation of rs10830963 genotype distribution and the metabolic parameters of pregnant women. The results showed a significant association of plasma glucose level with rs10830963 genotype distribution, especially for the FPG level (P<0.001 in dominant, recessive,

Table 1 Clinical and laboratory parameters of GDM and NP groups

Parameter	NP (n=536)	GDM (n=320)	P value
Gestational age (years)	30.32±4.07	32.83±4.79	<0.001*
Pre-pregnancy BMI (kg/m^2)	20.76±2.12	21.35±2.44	<0.001*
Weight-gain during pregnancy (kg)	16.65±0.61	14.41±0.62	0.012*
Systolic pressure (mmHg)	117.22±11.23	117.58±11.66	0.621
Diastolic pressure (mmHg)	75.71±8.33	75.82±8.02	0.350
FPG OGTT (mmol/L)	4.49±0.30	5.11±0.71	<0.0001*
1-h OGTT (mmol/L)	7.41±1.29	9.73±1.85	<0.0001*
2-h OGTT (mmol/L)	6.54±1.00	8.55±1.57	<0.0001*
TG (mmol/L)	3.19±0.16	3.94±0.30	0.022*
TC (mmol/L)	5.87±1.18	5.62±1.09	0.001*

GDM: gestational diabetes mellitus; NP: normal pregnancy; BMI: body mass index; FPG: fasting plasma glucose; OGTT: oral glucose tolerance test; TG: triglyceride; TC: total cholesterol. 1 mmHg=0.133 kPa. All data are presented with mean±standard deviation (SD). * P<0.05.

Table 2 Genotype and allele frequencies of rs10830963 in GDM and NP groups

rs10830963	NP (n=536)#	GDM (n=320)#	OR (95% CI)	P value	HWE
Allele					
CC	175 (33%)	68 (21%)			0.732
CG	259 (48%)	171 (53%)			
GG	102 (19%)	81 (25%)			
C	609 (57%)	307 (48%)			
G	463 (43%)	333 (52%)	1.427 (1.413–1.441)	0.000**	
Genotype model					
Dominant			1.818 (1.290–2.562)	0.001*	
Recessive			1.459 (1.024–1.648)	0.036*	
Additive			2.085 (1.355–3.209)	0.001*	

Data are expressed as number (percentage). * P values were adjusted for age and body mass index; ** P value for allelic gene. GDM: gestational diabetes mellitus; NP: normal pregnancy; OR: odd ratio; CI: confidence interval; HWE: Hardy-Weinberg equilibrium.

and additive models). Furthermore, the distribution of rs10830963 genotype was also significantly associated with the 1-h (P<0.05 in dominant, recessive and additive models) and 2-h plasma glucose levels (P<0.05 in additive model) after drinking the glucose solution (Table 3).

2.4 Expression of MT2 receptor in placental tissues

In order to explore whether the MT2 receptor was expressed in the placenta, our study measured the protein expression of this receptor in the placental tissues by immunofluorescence. Co-localization by immunofluorescence was used to identify the expression of MT2 (red) in the trophoblast, marked by cytokeratin 7 (CK7, green). We observed that MT2 staining was strongly positive in CK7-positive cells, and MT2 expression was markedly increased in the placental tissue of the GDM group (Figs. 1a–1d). Furthermore, western blot showed that MT2 expression was significantly elevated in the GDM group, as compared with the NP group (Figs. 1e and 1f). *MTNR1B* messenger RNA (mRNA) expression in the GDM group was also significantly higher than that in the NP group (Fig. 1g).

2.5 Effects of MLT on the expression of GLUTs, MT2 receptor, and PPARγ

In order to understand the effect of MLT signaling on glucose uptake and transport, HTR-8/SVneo cells were treated with MLT (0.25, 0.5, and 1.0 mmol/L) or received no treatment (dimethyl sulfoxide (DMSO)). Quantification showed that glucose consumption was significantly increased in MLT-treated (0.25, 0.5, and 1.0 mmol/L) cells compared with the control group (Fig. 2a). Moreover, GLUT4 protein expression also significantly increased after incubation with 1.0 mmol/L MLT compared with the control group. However, for the GLUT1 and GLUT3 transporters, no significant change in protein expression could be observed (Figs. 2b and 2d).

In order to explore the mechanism of MLT in regulating glucose uptake and transport, we measured the expression of MT2, a high-affinity receptor for MLT and PPARγ, which is key regulator of glucose uptake and transport. We found that the expression levels of MT2 and PPARγ proteins were significantly increased after incubation with 1.0 mmol/L MLT compared with the control group (Figs. 2c and 2e). Taken together, these findings suggested that the MLT-induced increases in glucose uptake and transport are likely due to the MT2 receptors and PPARγ.

2.6 Inhibition effects of MLT signaling on glucose transport and PPARγ expression by knockdown of the MT2 receptor

In order to determine whether MT2 is a key receptor mediating MLT signaling in the regulation of glucose uptake and transport, HTR-8/SVneo cells were transfected with small interfering RNAs (siRNAs) (si-MT2 and si-NC) in order to silence the expression of MT2 receptor combined with 1.0 mmol/L MLT (Fig. 3a). We analyzed the effectiveness of the three siRNAs (siRNA1, siRNA2, and siRNA3), and siRNA3 was the most effective in silencing MT2 expression (Figs. 3b and 3c). Then, we measured the alterations of glucose uptake in cells and the expression of GLUTs. We observed that the glucose consumption of si-MT2+1.0 mmol/L MLT group was significantly decreased compared with the si-NC+1.0 mmol/L MLT group

Table 3 Relationships between rs10830963 and relative metabolic parameters

Parameter	Genotype			*P* value		
	CC (*n*=243)	GC (*n*=430)	GG (*n*=183)	Dominant model	Recessive model	Additive model
Weight-gain during pregnancy (kg)	15.32±3.38	15.17±4.54	14.64±4.24	0.895	0.574	0.854
FPG OGTT (mmol/L)	4.63±0.50	4.78±0.63	4.91±0.62	0.000	0.000	0.000
1-h OGTT (mmol/L)	8.18±1.85	8.45±1.99	8.76±1.81	0.029	0.011	0.003
2-h OGTT (mmol/L)	7.26±1.65	7.41±1.59	7.64±1.65	0.144	0.060	0.033
TG (mmol/L)	4.16±1.89	4.49±3.46	3.31±1.04	0.325	0.319	0.235
TC (mmol/L)	5.79±1.08	5.83±1.16	5.71±1.09	0.577	0.211	0.262
Neonatal birthweight (kg)	3.29±0.40	3.23±0.38	3.27±0.42	0.232	0.644	0.156
Neonatal plasma glucose (mmol/L)	3.14±1.28	3.24±1.29	3.10±1.02	0.391	0.622	0.285

FPG: fasting plasma glucose; OGTT: oral glucose tolerance test; TG: triglyceride; TC: total cholesterol. All data are presented as mean± standard deviation (SD). *P* values were adjusted for age and body mass index.

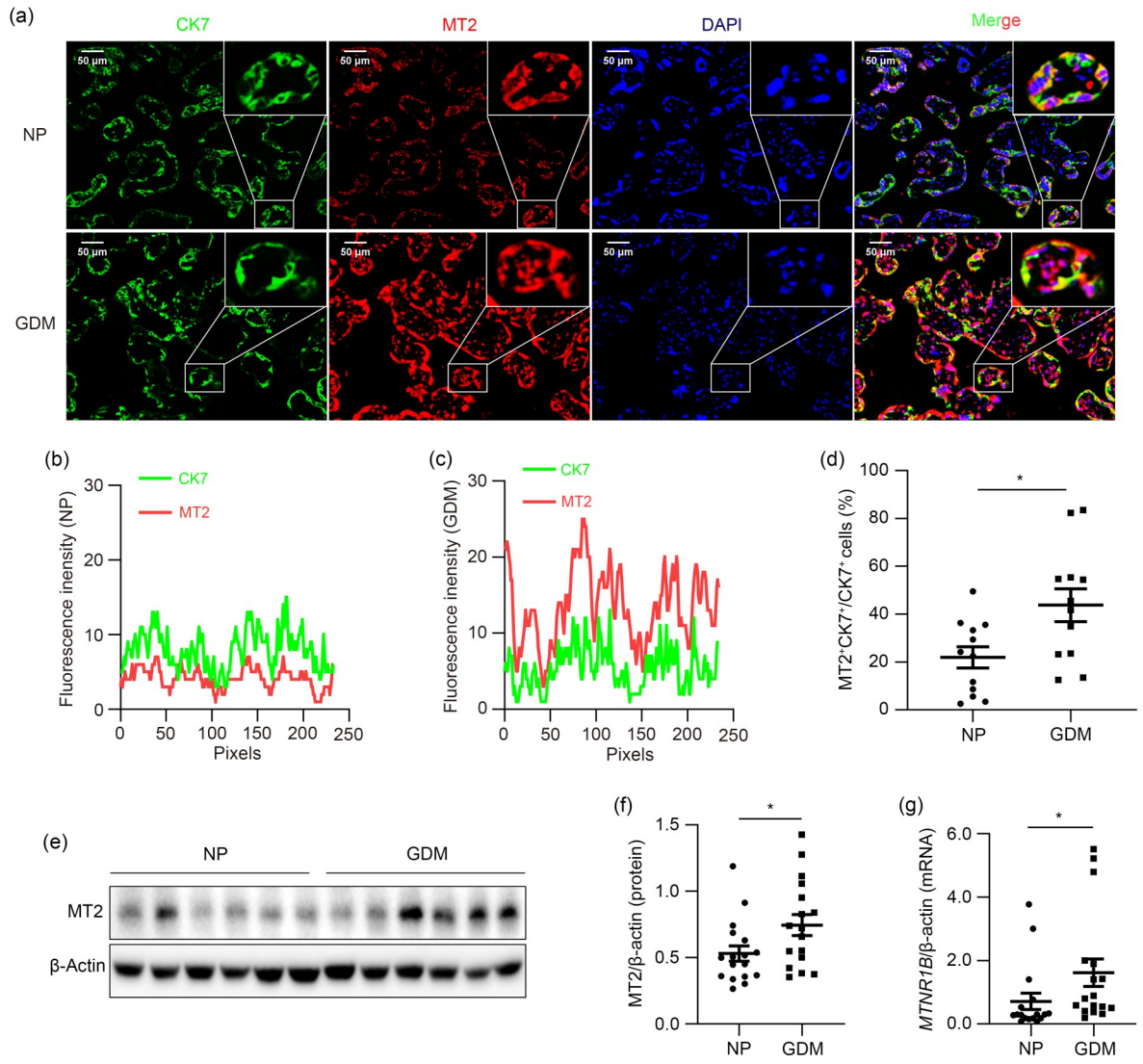

Fig. 1 Expression of MT2 receptor in the placental tissues of pregnant women in GDM and NP groups. (a–d) Representative images and quantitative analyses of immunofluorescence double staining of MT2 (red) and CK7 (green) in placental tissues of GDM (*n*=12) and NP (*n*=12) groups. (e, f) Representative images and quantitative analyses of MT2 protein expression detected by western blot in placental tissues of GDM (*n*=17) and NP (*n*=17) groups. (g) Quantitative analyses of *MTNR1B* mRNA expression in placental tissues of GDM (*n*=17) and NP (*n*=17) groups detected by quantitative real-time polymerase chain reaction (qPCR). GDM: gestational diabetes mellitus; NP: normal pregnancy; CK7: cytokeratin 7; MT2: melatonin receptor 1B; mRNA: messenger RNA; SEM: standard error of the mean. * *P*<0.05. (d, f, g) Data are expressed as mean±SEM.

(Fig. 3d). Furthermore, the expression levels of GLUT1 and GLUT4 were also significantly decreased in the si-MT2+1.0 mmol/L MLT group compared with the si-NC+1.0 mmol/L MLT group (Figs. 3e and 3f).

With the aim to further confirm that transcription factor PPARγ acts as a downstream of MT2 receptor in the regulation of GLUTs, we detected PPARγ protein expression. We observed that it was significantly decreased in the si-MT2+1.0 mmol/L MLT group compared with the si-NC+1.0 mmol/L MLT group (Figs. 3g and 3h). Overall, these findings suggested that the function of MLT signaling on the upregulation of glucose uptake and transport is mediated by MT2 receptors.

3 Discussion

This study revealed that the rs10830963 variant was significantly associated with GDM, as well as elevated FPG, 1-h and 2-h plasma glucose levels.

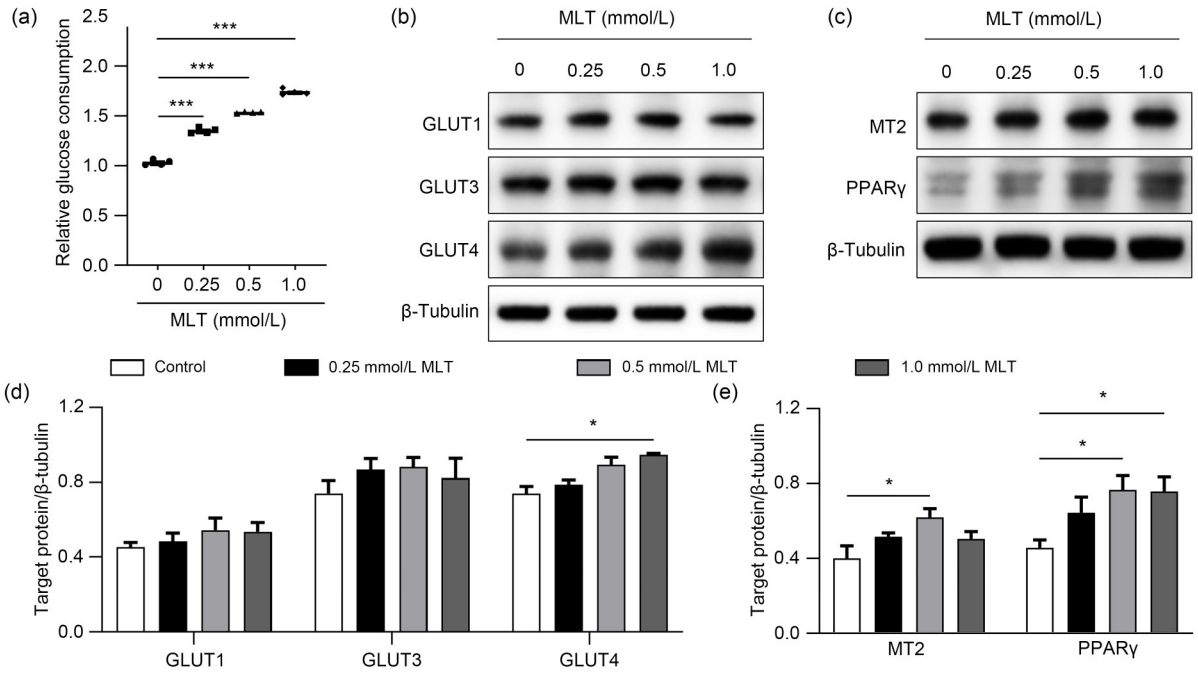

Fig. 2 Effects of MLT on the expression of GLUTs, MT2, and PPARγ. HTR-8/SVneo cells were incubated with MLT gradient concentrations consisting of 0, 0.25, 0.5, and 1.0 mmol/L. (a) Relative quantitative analyses of glucose consumption (*n*=4). (b, d) Representative western blot images and quantitative analyses of GLUT1, GLUT3, and GLUT4 protein expression among the four groups (*n*=6). (c, e) Representative western blot images and quantitative analyses of MT2 and PPARγ protein expression among the four groups (*n*=6). Values are expressed as mean±SEM. MLT: melatonin; GLUT: glucose transporter; MT2: melatonin receptor 1B; PPARγ: peroxisome proliferator-activated receptor γ; SEM: standard error of the mean. * *P*<0.05, * *P*<0.001**

Studies in Saudi, Mexican Americans, and Greek populations also identified a relationship between the rs10830963 variant and GDM susceptibility (Vlassi et al., 2012; Ren et al., 2014; Alharbi et al., 2019). Besides, the rs10830963 variant was also associated with offspring's birth weight (Beaumont et al., 2018). The variant of another locus of *MTNR1B* gene, rs10830962, significantly interacts with the offspring's obesity (Liang et al., 2020). Notably, some studies proposed that the effect of maternal genotype on fetal metabolic disorders such as high birth weight is due to the high glucose intrauterine environment, rather than through shared alleles with the fetus (Beaumont et al., 2018; Zhou et al., 2022). Raised maternal glucose leads to elevated placental glucose transfer and fetal insulin secretion, resulting in higher offspring's birth weight (Haggarty et al., 2002). Therefore, understanding the maternal determinants of fetal growth could be beneficial for reducing the prevalence of metabolic disorders in offspring.

We found that carrying the rs10830963 G risk allele significantly increased the occurrence of GDM, as compared with carrying the C risk allele. The rs10830963 is localized in the 5' promoter region of the *MTNR1B* gene, which may result in alterations of mRNA and protein expression (Karamitri and Jockers, 2019). In the placenta of GDM, the expression of MT2 protein in the carriers of the G allele was also significantly higher than that in the carriers of the C allele (Li et al., 2019), suggesting that the variant of rs10830963 may contribute to the development of GDM by altering of transcription and protein expression of MTNR1B. In this study, elevated expression levels of *MTNR1B* mRNA and protein were observed in GDM placenta, and this difference was predominantly exhibited in trophoblast cells. Therefore, we assumed that the increased fetal glucose uptake may be related to the alteration of MT2 receptor expression in the placenta.

MT2 is a high-affinity receptor for MLT expressed at central and peripheral sites. Despite the low levels of MT2 expression in peripheral tissues, direct effects of MLT on specific tissue functions, such as glucose uptake and transport, glycogen synthesis, and glycolysis, have been reported (Ha et al., 2006; Li et al., 2018). In this study, we found that MLT upregulated

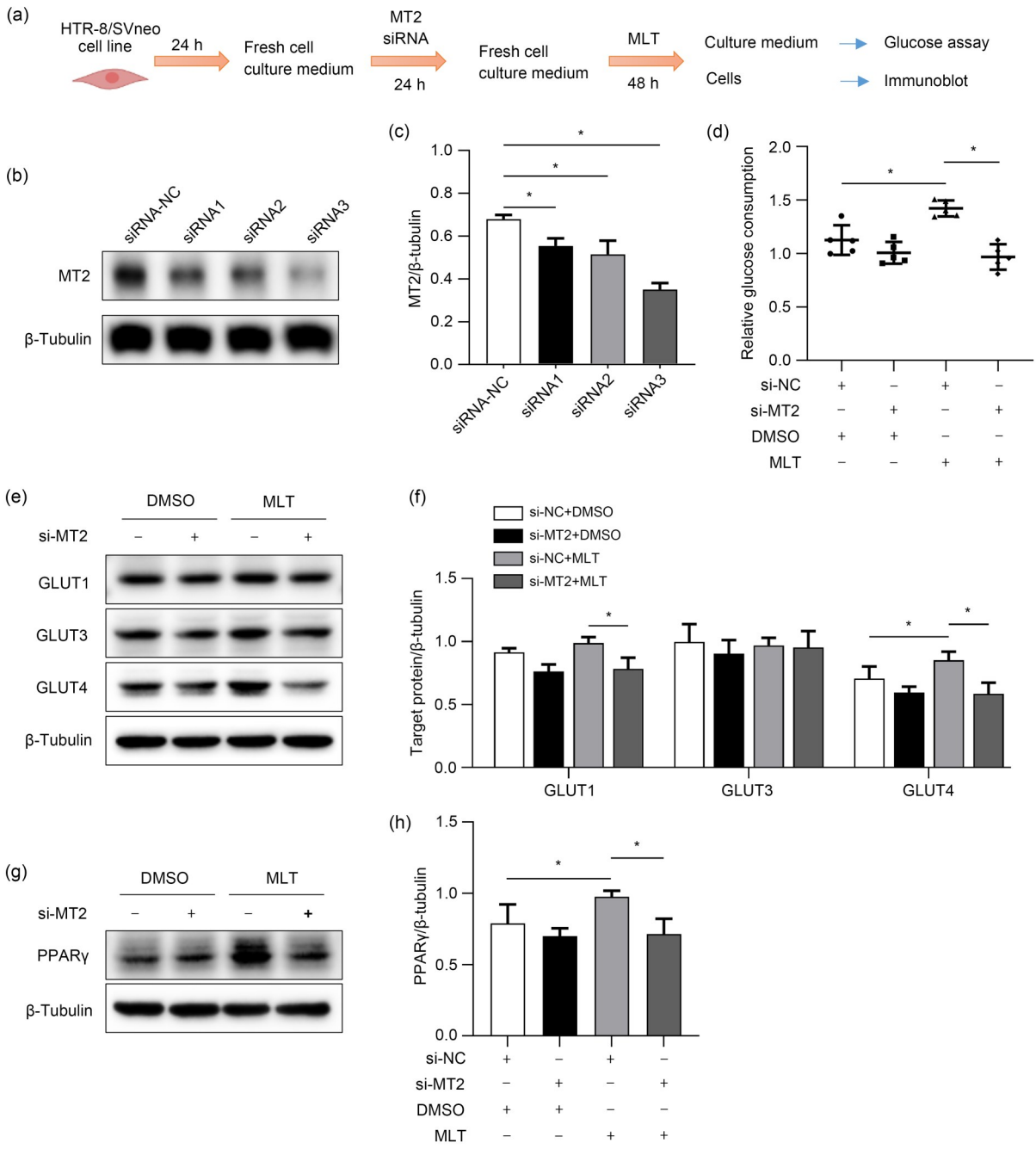

Fig. 3 Effects of MLT on the expression of GLUTs and PPARγ via MT2 receptor. HTR-8/SVneo cells were treated with MT2 receptor knockdown (si-MT2) or not (si-NC) for 24 h, and then were incubated with 1.0 mmol/L MLT or not (DMSO) for 48 h. (a) Schematic diagram of the experimental procedure. (b, c) Representative western blot images and quantitative analyses of MT2 protein expression (n=3). (d) Relative quantitative analyses of glucose consumption (n=5). (e, f) Representative western blot images and quantitative analyses of GLUT1, GLUT3, and GLUT4 protein expression (n=6). (g, h) Representative western blot images and quantitative analyses of PPARγ protein expression (n=6). Values are expressed as mean±SEM. MLT: melatonin; DMSO: dimethyl sulfoxide; GLUT: glucose transporter; MT2: melatonin receptor 1B; PPARγ: peroxisome proliferator-activated receptor γ; SEM: standard error of the mean. * P<0.05.

the uptake and transport of glucose in HTR-8/SVneo cells via the MT2 receptor. The placenta mediates maternal-fetal nutrient transport, and the transportation of glucose provides energy for fetal growth and metabolism. However, extra glucose transferring to fetus may lead to fetal hyperglycemia or macrosomia. Thus, we

speculated that the MLT signaling pathway mediated by the MT2 receptor may be involved in the mechanism of fetal hyperglycemia and macrosomia in GDM.

The transportation of glucose in the placenta is mediated by GLUTs. GLUT1 is a universal transporter with approximately 3-fold higher density in MVM than in BM, while GLUT1 expression in BM increases to approximately 2-fold in the second and the third trimesters, and remains at that level until term (Illsley and Baumann, 2020). The expression of GLUT3 is primarily in MVM, and it decreases significantly with increasing gestational age (Brown et al., 2011). GLUT4 is expressed in the cytosol of syncytial cells in the first trimester, which can be recruited rapidly to the cell surface under insulin stimulation. The expression of GLUT4 significantly decreased in the third trimester (Illsley and Baumann, 2020). GLUT4 has long been known as an insulin-responsive GLUT; however, some studies found that its effect on glucose homeostasis can also be independent of insulin. Muscle-specific insulin receptor knockout mice exhibit enlarged fat mass and greatly decreased glucose uptake level when stimulated by insulin, but the muscle glycogen level remains normal (Brüning et al., 1998). This indicates a possible insulin-independent pathway for glucose transport, such as adiponectin and leptin (Xue and Kahn, 2006). Our study revealed that MLT can also significantly increase glucose uptake and GLUT4 protein expression independent of insulin stimulation. Nonetheless, few studies have focused on the effect of MLT on glucose transport under insulin-independent pathways, prompting further research in this field.

Yao et al. (2017) reported that GLUT1 expression is increased in the placenta of pregnant women with GDM, and GLUT1 mRNA and protein expression levels are positively correlated with the birth weight of infants from GDM women with macrosomia. In the placenta of pregestational diabetes and insulin-controlled GDM women, GLUT4 expression was positively correlated with the birth weight of corresponding infants (Stanirowski et al., 2019). Based on the above studies, we formulated the following hypothesis: the increase in GLUTs in the placenta of GDM leads to the enhanced placental ability of glucose uptake and transport, which creates a high-glucose environment for the offspring, resulting in several adverse fetal outcomes. Therefore, we considered it highly important to explore the related pathways of GLUT expression.

Glucose transport is regulated by various signaling pathways. Our study indicated that MLT stimulated glucose uptake and the expression of GLUT4 protein through MT2 receptors in HTR-8/SVneo cells, and PPARγ expression was also increased. PPARγ is a key regulator of glucose uptake and transport, and acts to increase insulin sensitivity and glucose metabolism (Liao et al., 2007; Montaigne et al., 2021). Thus, we assumed that MLT affects the uptake and transport of glucose by modulating the expression of PPARγ, the mechanism of which needs to be elucidated by further research.

The present study has some limitations that should be addressed. First, the expression levels of GLUTs were not detected in GDM and NP tissues. Second, although we observed that the effect of MLT on GLUT4 expression can be independent of insulin stimulation, we still need to explore the effect of MLT on GLUT4 in the presence of insulin due to increased insulin secretion in early pregnancy. Third, the regulation of glucose uptake and the expression of GLUTs by PPARγ in trophoblasts requires further investigation. Fourth, the function of MT2 receptor in regulating glucose uptake and transport in vivo is yet to be explored.

4 Conclusions

To summarize, this study confirmed that the genetic polymorphism of rs10830963 contributed to high plasma glucose levels (FPG, 1-h and 2-h plasma glucose) and GDM. The MT2 receptor encoded by *MTNR1B* genes was predominantly expressed by trophoblasts in the placenta and was increased in GDM. In vitro, MLT upregulated glucose uptake and the expression of GLUT4 via MT2 receptor in trophoblasts, which may be mediated by the transcriptional factor PPARγ. These findings suggest that the *MTNR1B* rs10830963 variant is a potential risk factor for developing GDM. The altered expression of MT2 receptor in the placenta of GDM may be involved in the change of placental glucose uptake and transport levels, which could be associated with abnormal glucose metabolism and macrosomia in children born

to GDM mothers. However, large-scale prospective studies are still needed to determine the relationship between the rs10863963 variant and GDM susceptibility. In addition, the underlying mechanism for the association of this variant and the development of GDM and maternal and fetal adverse outcomes remains to be explored.

Materials and methods

Detailed methods are provided in the electronic supplementary materials of this paper.

Acknowledgments

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Author contributions

Lijie WEI performed the experiments and prepared this manuscript. Peng GAO, Jingyi ZHANG, and Xuan ZHOU were in charge of obtaining written consent from participants, collecting clinical and biochemical data, and confirming the data accuracy. Huiting ZHANG, Yi JIANG, and Yuanyuan DU participated in experimenting. Xuan GAO, Yuting CHEN, and Jiaqi LI were responsible for data analysis and results discussion. Shenglan ZHU, Chenyun FANG, and Mengzhou HE proofread and adjusted the manuscript. Shaoshuai WANG, Ling FENG, and Jun YU conceptualized and provided methodology opinions. All authors have read and approved the final manuscript, and therefore, they have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Lijie WEI, Yi JIANG, Peng GAO, Jingyi ZHANG, Xuan ZHOU, Shenglan ZHU, Yuting CHEN, Huiting ZHANG, Yuanyuan DU, Chenyun FANG, Jiaqi LI, Xuan GAO, Mengzhou HE, Shaoshuai WANG, Ling FENG, and Jun YU declare that they have no conflict of interest.

This study protocol was approved by the Tongji Hospital, Tongji Medical College of Huazhong University of Science and Technology Ethics Committee (No. TJ-IRB20170506), and was performed under the Declaration of Obstetrics Department. Informed consent was obtained from all patients for being included in the study. Additional informed consent was obtained from all patients for whom identifying information is included in this article.

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Supplementary information

Materials and methods